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Applicant (s)

(21) Patentansökningsnummer 9801148-9
Patent application number

(86) Ingivningsdatum 1998-03-31
Date of filing

(30) Prioritet begärd från 97-10-14 SE 9703745-1

Stockholm, 1998-09-17

För Patent- och registreringsverket
For the Patent- and Registration Office


Asa Dahlberg

Avgift
Fee 170:-

Ink. t. Patent- och reg.verket

1998 -03- 31

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NEW RECEPTORS

FIELD OF THE INVENTION

5 The present invention relates to novel vitamin D receptor related (VDRR) polypeptides. Nucleic acid sequences encoding the same, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention, and uses thereof.

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BACKGROUND OF THE INVENTION

Nuclear hormone receptors is a large group of conditionally regulated transcription factors. These receptors are activated and regulate target gene expression in response to binding a variety of small chemical molecules (ligands) including steroids, vitamin D3, retinoids, eicosanoides (prostanoids), thyroid hormone and cholesterol derivatives.

15 A growing number of structurally related receptors have been identified for which no ligands yet have been identified. This group of receptors is referred to as orphan nuclear receptors (ONRs). A review of the ONRs can be found in Enmark et al, Mol. Endo., vol. 10, No. 11 (1996) pp. 1293-1307, which is hereby incorporated by reference. The pivotal importance of a number of ONRs for processes such as metabolic homeostasis, cell differentiation and development have been demonstrated both by biochemical and genetic techniques. In addition, several ONRs have also been implicated as key factors in a variety of common diseases and disorders such as diabetes, obesity, inflammatory conditions and proliferative diseases.

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Based on these findings it is generally believed that novel ONRs are going to become potential drug targets for therapeutic invention of common diseases. Thus, it is of great importance to identify such receptors.

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SUMMARY OF THE INVENTION

The present invention relates to novel vitamin D receptor related (VDRR) polypeptides, and formulations containing the same. Nucleic acid sequences encoding the VDRR polypeptides, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention. The invention further relates to VDRR polypeptides for use as medicaments, and use of substances affecting VDRR signal transduction for the manufacture of medicaments for treating metabolic, proliferative or inflammatory conditions. The present invention also relates to methods for identifying clones encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods for identifying substances for treatment of conditions affected by a VDRR polypeptide. More specifically, the novel VDRR polypeptide can be the polypeptide designated VDRR γ , which may be regulated by any small chemical molecule similar in structure to known ligands for nuclear receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 - The cDNA sequence encoding the novel nuclear receptor polypeptide vitamin D receptor related gamma (VDRR γ) is shown.

Figure 2 - Evolutionary neighbor-joining tree for VDRR γ as given by DBD-HMM alignment.

Figure 3 - Evolutionary neighbor-joining tree for VDRR γ as given by LBD-HMM alignment.

Figure 4 - The deduced amino acid sequence of VDRR γ is shown.

Figure 5 - Expression of VDRR γ in adult human tissues. The numbers on the right hand side, refer to kilobasepairs of the mRNA.

Figure 6 - Vitamin D3 transactivate a GAL4-DBD/VDR-LBD fusion protein but not a GAL4-DBD/VDRR γ -LBD fusion protein in transient transfections of CV-1 cells. The number on the left hand side refer to relative luciferase activity of the GAL4-luciferase reporter gene.

Figure 7 - The cDNA sequence encoding VDRR γ -2 with an alternatively spliced 5'-end compared to VDRR γ is shown.

Figure 8 - The deduced amino acid sequence of VDRR γ -2 is shown.

Figure 9 - Heterodimerization of VDRR γ with a retinoid X receptor (RXR) is shown.

5 Figure 10 - The effect of pregnenolone derivatives as activators of VDRR γ are shown.

Figure 11 - The effect of pregnenolone 16 α -carbonitrile (PCN), dexamethasone and an antiprogesterin (RU486) as activators of VDRR γ are shown.

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DETAILED DESCRIPTION OF THE INVENTION

The objects above are met by the present invention, which relates to an isolated or recombinant nucleic acid comprising a contiguous nucleic acid sequence encoding a vitamin D receptor related (VDRR) polypeptide. The VDRR polypeptide is suitably of mammalian, preferably human, origin.

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In preferred embodiments of the present invention, the nucleic acid encoding the VDRR polypeptide contains a DNA-binding domain (DBD) comprising about 77 amino acids with 9 cysteine residues. The DBD is further characterized by the following amino acid sequence identity relative to the DBDs of human Vitamin D Receptor (hVDR) and

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Orphan Nuclear Receptor 1 isolated from *Xenopus laevis* (xONR1), respectively:

(i) at least about 50% amino acid sequence identity with the DBD of hVDR, suitably at least 60% amino acid sequence identity with the DBD of hVDR; and

(ii) at least about 55% amino acid sequence identity with the DBD of xONR1, suitably at least 65% amino acid sequence identity with the DBD of xONR1.

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More particularly, the amino acid sequence identity relative to the DBDs of hVDR and xONR1, respectively is

(i) about 65% amino acid sequence identity with the DBD of hVDR; and

(ii) about 71% amino acid sequence identity with the DBD of xONR1.

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In preferred embodiments of the present invention, the nucleic acid encoding the VDRR polypeptide contains a ligand-binding domain (LBD) characterized by the following amino acid sequence identity, relative to the LBDs of hVDR and xONR1, respectively:

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(i) at least about 30% amino acid sequence identity with the LBD of hVDR, suitably at least 35% amino acid sequence identity with the LBD of hVDR; and

(ii) at least about 40% amino acid sequence identity with the LBD of xONR1, suitably at least 45% amino acid sequence identity with the LBD of xONR1.

5 More particularly, the amino acid sequence identity relative to the LBDs of hVDR and xONR1, respectively is

(i) about 42% amino acid sequence identity with the LBD of hVDR; and

(ii) about 54% amino acid sequence identity with the LBD of xONR1.

10 In particularly preferred embodiments, the nucleic acid sequences of the present invention are those given in Fig. 1 or Fig. 7.

The present invention also relates to a nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR polypeptide in a sample. Suitably, the probe comprises at least 14 contiguous nucleotides, and preferably at least 28 contiguous nucleotides, of the nucleic acid sequences given in Fig. 1 or Fig. 7. The nucleic acid probe can be
15 used in a method for identifying clones encoding a VDRR polypeptide, wherein the method comprises screening a genomic or cDNA library with the probe under low stringency hybridization conditions, and identifying those clones which display a substantial degree of hybridization to said probe.

The present invention further relates to an isolated or recombinant VDRR polypeptide. The polypeptide can be full-length, at which the sequence of amino acids is identical to the corresponding sequence found in mammals in general, and in human beings in particular. In the present invention, the polypeptide can also be a truncated, extended or mutated form of the full-length polypeptide. Truncated and extended forms relate to VDRR polypeptides where one or more amino acids are missing or have been added, respectively,
20 at the N terminal end of the polypeptide chain. Mutated forms relate to VDRR polypeptides where one or more amino acid has been substituted by another amino acid. Suitably, the isolated or recombinant VDRR polypeptide exhibits the amino acid sequences given in Fig. 4 or Fig. 8.

25 The N-terminal sequence of the present nucleic acids encoding VDRR polypeptides, as well as the amino acid sequence of the present VDRR polypeptides, may vary. Thus, various N-terminal isoforms are envisaged, e.g. any of $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\gamma 1$ or $\gamma 2$ as
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disclosed in Fig. 7B of Transcription Factors 3: nuclear receptors, Protein Profile, vol. 2, issue 11 (1995), pp. 1173-1235. This review of nuclear receptors generally is hereby incorporated by reference. More specifically, Vitamin D receptors and related orphans, e.g. ONR1, are discussed at p. 1191-1992.

5 The present invention further relates to pharmaceutical formulations comprising an isolated or recombinant VDRR polypeptide, and one or more therapeutically acceptable excipients. Examples of excipients that can be used are carbohydrates, e.g. monosaccharides, disaccharides and sugar alcohols, such as saccharose and sorbitol. Further examples include amino acids, e.g. histidine and arginine, surfactants, e.g. polyoxyethylene sorbitan fatty acid
10 esters, inorganic salts, e.g. sodium chloride and calcium chloride, and complexing agents, e.g. EDTA and citric acid.

The present formulation can be in the form of an aqueous solution ready-for-use, or dried, particularly lyophilized. In the latter case, the formulation is reconstituted with a liquid, e.g. sterile water or saline, before use.

15 The present invention further relates to an expression vector comprising an isolated or recombinant nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide. The invention also relates to a cell containing such an expression vector.

The present invention further relates to a cell containing an isolated or recombinant
20 nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide.

The present invention further relates to a process for recombinant production of a VDRR polypeptide, by expressing an isolated or recombinant contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide in a suitable host cell,
25 preferably an eukaryotic cell.

The present invention further relates to method for identifying a ligand to a VDRR, e.g. by a cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay. It also relates to a method for identifying a substance for treatment of a condition affected by a VDRR polypeptide, comprising screening for an agonist or an antagonist of
30 VDRR polypeptide signal transduction to be used for treating metabolic, proliferative or inflammatory conditions.

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The present invention further relates to a VDRR polypeptide for use as a medication, as well as use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions. More particularly, the present invention can be used for the manufacture of medicaments for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesteremia or hyperlipoproteinemia. The present invention can be used also for the manufacture of medicaments for treating osteoporosis, rheumatoid arthritis, benign and malignant tumors, hyperproliferative skin disorders or hyperparathyroidism.

The present invention further relates to a method for treating metabolic, proliferative or inflammatory conditions by introducing into a mammal a nucleic acid vector encoding for expression of a VDRR polypeptide. The nucleic acid vector is capable of transforming a cell *in vivo* and expressing said polypeptide in said transformed cell.

The present invention further relates to a method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR signal transduction, specifically a VDRR polypeptide.

In the present invention, the term "isolated" in connection with VDRR polypeptides or nucleic acids encoding the same, relates to nucleic acids or polypeptides that have been isolated from a natural source, e.g. the liver, small intestine or colon of a human being. The isolated VDRR polypeptides or nucleic acids of the present invention are unique in the sense that they are not found in a pure or separated form in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free environment or in a different cellular environment. The term does not imply that the sequence is the only nucleic acid or amino acid sequence present, but that it is the predominant nucleic acid or amino acid sequence present. Furthermore, the nucleic acid or polypeptide should be essentially free of non-amino acid or non-nucleic acid material naturally associated with the respective product. In this context, essentially free relates to more than 80%, suitably more than 90%, and preferably more than 95% purity.

The inventors of the present invention, have surprisingly isolated a novel nucleic acid sequence, and a polypeptide encoded by said nucleic acid sequence. Thus, a novel cDNA encoding a polypeptide designated VDRRy has been cloned and characterized. This poly-

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peptide is, based on amino acid sequence similarity, a novel member of the nuclear (hormone) receptor supergene family. Hidden Markov Models (HMMs) in combination with phylogenetic analysis such as neighbor-joining tree methods and other statistical algorithms shows that VDRR γ belong to a sub-family of vitamin D receptors (VDRs) and a VDR-like receptor from *Xenopus laevis* designated xONR1 (see Smith et al., Nucl. Acids Res., 22 (1994), No. 1, pp. 66-71). The VDRR γ , therefore, is one member of a family of Vitamin D receptor related (VDRR) polypeptides.

This finding, in combination with the highly restricted expression pattern we observe for human VDRR γ (liver, small intestine and mucosa of colon) and in analogy to other nuclear receptors exhibiting a tissue specific expression pattern such as the peroxisome proliferator-activated receptors (PPARs) - suggest that VDRR γ performs important physiological functions in liver, small intestine and colon. Accordingly, VDRR γ is likely to be an important sensor of key metabolic pathways affecting lipid, carbohydrate or amino acid metabolism/homeostasis. In addition, the highly selective tissue specific expression pattern suggest that VDRR γ may participate in cellular differentiation and development of these tissues.

An additional human VDRR γ cDNA with an alternatively spliced 5'-end has been identified (see Fig. 7). The VDRR γ cDNAs are thus able to encode at least one alternative N-terminal variant (Fig. 8) in addition to the VDRR γ polypeptide shown in Fig. 4. In analogy to other members of the nuclear receptor supergene family such as ROR α and RAR α these N-terminal isoforms of VDRR γ may specify different functions including DNA-binding specificity and/or promoter specific activation (Gronemeyer and Laudet, 1995).

In the present specification, the term VDRR γ relates to the various polypeptides corresponding to the differentially spliced VDRR γ cDNAs including VDRR γ -1 and VDRR γ -2. However, when reference is made to Fig. 1 and Fig. 4, VDRR γ cDNA and VDRR γ relates specifically to VDRR γ -1 cDNA and VDRR γ -1, respectively. In the same way, when reference is made to Fig. 7 and Fig. 8, VDRR γ cDNA and VDRR γ relates specifically to VDRR γ -2 cDNA and VDRR γ -2, respectively.

In contrast to the VDRR γ -2 cDNA, the VDRR γ -1 cDNA does not contain a classical AUG initiation codon but instead may initiate at an alternative CUG codon. This putative

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non-AUG start site is located in a favorable sequence context for efficient initiation from alternative start sites and is in frame with the entire open reading frame and preceded by a stop codon.

5 Taken together, the VDRRs in general, and more specifically the VDRR γ , may be important in

- 1) metabolic diseases such as obesity, diabetes (type I and II), lipoprotein disorders,
- 2) proliferative conditions such as tumors (benign and malignant) of the small intestine and colon,
- 10 3) ulcero-inflammatory diseases of small intestine and colon such as Crohn's disease and ulcerative colitis, and
- 4) congenital anomalies of small intestine and colon.

The high amino acid sequence identity of VDRR γ with the VDR both in the DNA-binding domain (DBD) and ligand-binding domain (LBD) indicate that these two receptors may also have overlapping yet distinct functional characteristics. In analogy, retinoic acid
15 receptors (RARs) and retinoid X receptors (RXRs) have similar amino acid sequence identities in the DBD and LBD region as the VDR and VDRR γ . RARs and RXRs have been shown to have distinct functional similarities such that both receptors bind 9-cis retinoic acid and have overlapping DNA-binding specificities and accordingly regulate overlapping gene networks. Based on these findings, VDRR γ may be regulated by small chemical molecules
20 similar in structure to known ligands for nuclear receptors but not necessarily identical to ligands for the 1 α , 25-dihydroxy vitamin D₃ receptor. Furthermore, VDRR γ may regulate vitamin D₃ responsive gene networks by binding to a Vitamin D responsive element (VDRE)-like DNA sequence. In the present application, the 1 α , 25-dihydroxy vitamin D₃ receptor is abbreviated as the Vitamin D receptor (VDR).

25 In the present invention, the substance affecting VDRR signal transduction can be any small chemical molecule of natural or synthetic origin, e.g. a carbohydrate such as an aromatic compound. The small molecule may have a molecular weight in the range of from about 100 up to about 500 Da. Suitably, the small chemical molecule has a molecular weight in the range of from 200 up to 400 Da. Preferably, the small chemical molecule has a
30 molecular weight of about 300 Da.

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The human VDRR γ polypeptides, including VDRR γ -1 and VDRR γ -2, have been shown to be activated e.g. by pregnenolones and estradiol (weakly), but not by certain other steroid hormones such as cortisol, aldosterone, progesterone and estrogen, and most likely not by progestines and glucocorticoids. Thus, human VDRR γ is not activated by

5 pregnenolone 16 α -carbonitrile (PCN), a glucocorticoid antagonist. For this reason, human VDRR γ can also be designated human pregnenolone activated (nuclear) receptors (hPAR). Information about pregnenolone can be found e.g. in the Merck Index, 11th ed., Merck & Co., Inc. Rahway, N.J., USA, p. 7735, 1989.

10 Activators for human VDRR γ polypeptides, including VDRR γ -1 and VDRR γ -2, (hPAR-1 and hPAR-2, respectively), include but are not limited to pregnenolones, such as pregnane-ones, pregnane-diones, pregnane-triones, and pregnane-diols, and androstanes, such as androstane-ols, and androstane-diols. Suitably, the pregnenolones are non-planar, particularly 5 β -pregnanes.

15 Specific examples of activators and possibly ligands for human VDRR γ polypeptides, including VDRR γ -1 and VDRR γ -2, are the following compounds, which are marketed by Sigma-Aldrich of Sweden:

- i) 5 β -pregnane-3,20-dione
- ii) 3 α -hydroxy-5 β -pregnane-11,20-dione methanesulphonate
- iii) 5 β -pregnane-3 α ,20 β -diol
- 20 iv) pregnenolone
- v) Pregn-4-eno[16,17- δ][2]isoxazoline-3,20-dione, 6 α -methyl-3'-phenyl-, ethyl ether solvate
- vi) Pregna-1,4,9(11)-triene-3,20-dione, 21-[4-[6-methoxy-2-(4-morpholinyl)-4-pyrimidinyl]-1-piperazinyl]-16-methyl-, (16 α)-
- 25 vii) Estran-3-ol, 17-[[[3-(trifluoromethyl)phenyl]methyl]amino]-, (E)-2-butenedioate (1:1) (salt)
- viii) 9 α -Fluoro-5 α -androstane-11 β ,17 β -diol
- ix) Spiro[5 α -androstane-3,2'-benzothiazolin]-11-one, 17 β -hydroxy-17-methyl-
- x) Spiro[pregnane-3,2'-thiazolidine]-4'-carboxylic acid, 11 α -hydroxy-20-oxo-,
- 30 sodium salt
- xi) 17 β -Dimethylamino-17-ethynyl-5 α -androstane-11 β -ol

- xii) 6 β -Hydroxy-3,5-cyclo-5 α -pregnan-20-one, nitrite
xiii) 3 α -Hydroxy-5 β -pregnane-11,20-dione, acetate, 20-O-(methylsulfonyl)-oxime
xiv) 17 α -Methyl-5 α -androstane-11 β ,17-diol
xv) 5 β -Pregnane-3,11,20-trione, trioxime
5 xvi) 3 α -Hydroxy-5 β -pregnane-11,20-dione, 20-hydrazone with hydrazide of
1-(carboxymethyl) pyridinium chloride.

Genes coding for polypeptides, such as human vitamin D receptor related gamma (hVDRRy), may be cloned by incorporating a DNA fragment coding for the polypeptide into a recombinant DNA vehicle, e.g. a vector, and transforming suitable prokaryotic or eukaryotic host cells. Such recombinant DNA techniques are well known and e.g. described in
10 Methods in Enzymology, Academic Press, San Diego, CA, USA (1994), vols. 65 and 68 (1979), and vols. 100 and 101 (1983).

The host cells for use in the present invention can be prokaryotic or eukaryotic, preferably eukaryotic cells. Suitable eukaryotic host cells include but are not limited to cells
15 from yeast, e.g. Saccharomyces, insect cells and mammalian cells such as Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK), COS and the like. Suitable prokaryotic host cells include but are not limited to cells from Enterobacteriaceae, e.g. E. coli, Bacillus and Streptomyces.

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EXAMPLES

The following Examples are provided for purposes of illustration only and are not to be construed as in any way limiting the scope of the present invention, which is defined by the appended claims.

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EXAMPLE 1

Identification and isolation of human VDRRy cDNA

Expressed Sequence Tag (EST) databases were screened for nuclear receptor related sequences with a DNA-binding domain (DBD) profile of nuclear receptors. This search
30 profile was created by multiple alignment of a selected set of nuclear receptor sub-domains followed by a statistical calculation to obtain a so called Hidden Markov Model (HMM) of

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different subfamily members of the nuclear receptor supergene family. The cDNA of one of the nuclear receptor related EST sequences identified (Incyte clone no 2211526) was analyzed in detail by sequencing. After DNA sequencing of the entire Incyte cDNA clone (approximately 2200 basepairs) the clone was found to encode a putative ligand-binding domain (LBD) with 54% and 44% similarity to xONR-1 and to the vitamin D receptor (VDR), respectively. The cDNA of the Incyte clone was not full-length and did not encode a sequence corresponding to a complete DBD.

5'-RACE (rapid amplification of cDNA ends) of random primed cDNA from human liver RNA (InVitrogen) followed by cloning and DNA sequencing showed that the 5'-part of the cDNA corresponding to the Incyte clone encoded a DBD characteristic for nuclear receptors and with 71% and 65% sequence identity to xONR-1 and VDR, respectively. Multiple alignments in combination with evolutionary neighbor-joining tree analysis placed the polypeptide encoded by the cDNA (specified in Fig. 1) in the group of VDRs (Figs. 2 and 3) and was named human vitamin D receptor related gamma (VDRR γ). The deduced amino acid sequence of VDRR γ is given in Fig. 4.

EXAMPLE 2

Expression of VDRR γ mRNA in human tissues

Multiple tissue northern blots (Clontech) was used to determine the expression pattern of VDRR γ in adult human tissues. As shown in Fig. 5, VDRR γ is abundantly expressed in small intestine, mucosal lining of colon and liver but not in several other tissues including spleen, thymus, prostate, testis, ovary, peripheral blood leukocytes, heart, brain, placenta, lung, skeletal muscle, kidney and pancreas. To investigate if VDRR γ was expressed at lower levels in any of the other tissues examined, the filter was exposed for an extended time (one week as compared to overnight). Even after this prolonged exposure (data not shown), expression could still only be detected in the same tissues and not in any of the other tissues examined. The restricted expression pattern of VDRR γ suggest that this receptor is likely to have an important regulatory function in liver and intestine.

EXAMPLE 3

Transient transfections of GAL4-DBD/VDRR γ -LBD fusion protein using Vitamin D3

Transient transfections were performed to analyze if vitamin D3 activate the VDRR γ polypeptide. To this end, transient co-transfections of CV-1 cells were performed with expression plasmids encoding fusion proteins of the GAL4-DBD fused to the LBD of either the VDR or the VDRR together with a reporter-plasmid containing five GAL4 responsive elements upstream of the luciferase gene. After transfection, cells were treated with vehicle (DMSO) alone or with vitamin D3 for 48 hours followed by harvesting of the cells and measurement of the luciferase activity in cell extracts. As shown in Fig. 6, vitamin D3 (1 μ M) transactivate the GAL4-DBD/VDR-LBD but not the corresponding GAL4-DBD/-VDRR γ -LBD polypeptide under these conditions. This indicates that the two receptors may have distinct ligand-binding specificities.

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EXAMPLE 4

Identification and isolation of human VDRR γ cDNAs encoding multiple N-terminal isoforms

5'-RACE (see Example 1) of cDNA from human liver RNA followed by cloning and DNA sequencing identified an additional human VDRR γ cDNA with alternatively spliced 5'-end (see Fig. 7). The VDRR γ cDNAs are thus able to encode at least one alternative N-terminal variant (Fig. 8) in addition to the VDRR γ polypeptide shown in Fig. 4. The polypeptides disclosed in Fig. 4 and Fig. 8 which correspond to the differentially spliced VDRR γ cDNAs are designated as VDRR γ -1 and VDRR γ -2, respectively.

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EXAMPLE 5VDRR γ heterodimerise with RXR and bind to direct repeats (DRs) spaced by three or four nucleotides

- 5 Expression plasmids containing VDRR γ or RXR β cDNAs were transcribed using T7 polymerase and translated *in vitro* in TNT reticulocyte lysates (Promega, Madison, WI, USA). To investigate the DNA-binding specificity of VDRR γ a native gel mobility assay was employed essentially as described (Berkensam et al., Cell, 69, 401-412, 1992) in which *in vitro* translated VDRR γ was incubated in the presence or absence of *in vitro* translated
- 10 RXR β with different 32P-labelled direct repeats (DR-1 to DR-5) as indicated in Fig. 9. The direct repeats were derived from the DR-5 element in the RAR- β 2 promoter (de Thé et al., Nature, 343, 177-180, 1990) and modified to be separated by one to five nucleotides (Pettersson et al., Mechanisms of Dev., 54, 1-13, 1995). Protein-DNA complexes were separated on native 5% polyacryl-amide/0.25xTBE gels followed by autoradiography. As
- 15 shown in Fig. 9, of the five DRs tested efficient VDRR γ binding could only be detected with DRs separated by three or four nucleotides and only in the presence of RXR. However, weaker RXR-dependent binding could also be observed to DR-2 and DR-1 elements. These results demonstrate that VDRR γ require RXR heterodimerisation for efficient DNA-binding to a specific subset of DRs. These results, however, do not exclude the possibility that
- 20 VDRR γ may bind as a monomer, dimer or heterodimer to distinct but related DNA-sequences. Importantly, our results demonstrate that VDRR γ and other nuclear receptors including the VDR (e.g. Markose, E. R. et al., Proc. Natl. Acad. Sci. USA, 87, 1701-1705, 1990), THR α s (e.g. Gronemeyer, H. and Moras, D., Nature, 375, 190-191, 1995), LXR α s (e.g. Willy, P. J. et al., Genes. Dev., 9, 1033-1045, 1995), share common target DNA-sequence
- 25 specificity and thus may regulate overlapping gene networks.

EXAMPLE 6

Pregnenolone derivatives as activators of VDRR γ

For identifying activators or ligands for VDRR γ , a library of substances structurally
5 biased towards different classes of activators and ligands for nuclear receptors were tested.
The activation of VDRR γ was analyzed in a reporter gene assay in transiently Caco-2 (TC7)
cells (Carriere et al, 1994). In this initial screen, the synthetic substances with ability to
activate VDRR γ were found to be structurally similar to pregnenolones (data not shown).
Based on these results, naturally occurring pregnenolone derivatives were examined for
10 activation of VDRR γ . The results are shown in Fig. 10. As is evident from Fig. 10, VDRR γ
was activated about 5 to 12 fold by pregnenolone, 5 β -pregnane-3,20-dione, 5 β -pregnane-
3 α ,20 β -diol and 3 α -hydroxy-5 β -pregnane-11,20-dione methanesulphonate. In contrast to
the efficient activation observed by the 5 β -pregnane-3,20-dione, the corresponding planar
steroid derivative 5 α -pregnane-3,20-dione did not activate the receptor. Other 5 β -pregnanes
15 also activated VDRR γ efficiently as opposed to all planar pregnenolone derivatives tested, as
is also evident from Fig. 10.

EXAMPLE 7

20 Pregnenolone 16 α -carbonitrile (PCN), dexamethasone and an antiprogesterin (RU486) as activators of VDRR γ

Further experiments were performed to find out if pregnenolone 16 α -carbonitrile
(PCN), a glucocorticoid antagonist or dexamethasone are activators of VDRR γ . To this
effect, Caco-2 cells were transfected as before with VDRR γ and the activation was analyzed
25 after treatment of the cells with 10 μ M PCN or dexamethasone. The results are shown in
Fig. 11. As is evident from Fig. 11, VDRR γ was not activated by these substances, indicat-
ing that VDRR γ is not the human PCN receptor. This suggestion is corroborated by the
observation that also the antiprogesterin RU486 only caused a slight increase (two fold) in
VDRR γ mediated reporter gene activity as is evident from Fig. 11.

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CLAIMS

1. An isolated or recombinant nucleic acid comprising a contiguous nucleic acid sequence
5 encoding a vitamin D receptor related (VDRR) polypeptide.
2. The nucleic acid according to claim 1, wherein said VDRR polypeptide is of mammalian, preferably human, origin.
- 10 3. The nucleic acid according to claims 1 or 2, encoding the VDRR polypeptide containing a DNA-binding domain (DBD) comprising about 77 amino acids with 9 cysteine residues, wherein said DBD is further characterized by the following amino acid sequence identity relative to the DBDs of human Vitamin D Receptor (hVDR) and Orphan Nuclear Receptor 1 isolated from *Xenopus laevis* (xONR1), respectively:
15 (i) at least about 50% amino acid sequence identity with the DBD of hVDR; and
(ii) at least about 55% amino acid sequence identity with the DBD of xONR1.
4. The nucleic acid according to claim 3, wherein said DBD is characterized by the following amino acid sequence identity:
20 (i) at least 60% amino acid sequence identity with the DBD of hVDR; and
(ii) at least 65% amino acid sequence identity with the DBD of xONR1.
5. The nucleic acid according to claim 4, wherein said DBD is characterized by the following amino acid sequence identity:
25 (i) about 65% amino acid sequence identity with the DBD of hVDR; and
(ii) about 71% amino acid sequence identity with the DBD of xONR1.
6. The nucleic acid according to any previous claim, encoding the VDRR polypeptide, wherein the ligand-binding domain (LBD) of said polypeptide is characterized by the
30 following amino acid sequence identity, relative to the LBDs of hVDR and xONR1, respectively:

- (i) at least about 30% amino acid sequence identity with the LBD of hVDR; and
- (ii) at least about 40% amino acid sequence identity with the LBD of xONR1.

7. The nucleic acid according to claim 6, wherein said LBD is characterized by the following amino acid sequence identity:

- (i) at least 35% amino acid sequence identity with the LBD of hVDR; and
- (ii) at least 45% amino acid sequence identity with the LBD of xONR1.

8. The nucleic acid according to claim 7, wherein said LBD is characterized by the following amino acid sequence identity:

- (i) about 42% amino acid sequence identity with the LBD of hVDR; and
- (ii) about 54% amino acid sequence identity with the LBD of xONR1.

9. The nucleic acid according to any previous claim, wherein said nucleic acid sequence is that given in Fig. 1 or Fig. 7.

10. A nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR polypeptide in a sample.

11. The nucleic acid probe according to claim 10, wherein said probe comprises at least 14 contiguous nucleotides of the nucleic acid sequence given in Fig. 1 or Fig. 7.

12. A method for identifying clones encoding a VDRR polypeptide said method comprising screening a genomic or cDNA library with a nucleic acid probe according to claims 10 or 11 under low stringency hybridization conditions, and identifying those clones which display a substantial degree of hybridization to said probe.

13. An expression vector comprising a nucleic acid according to claim 1.

14. A cell containing a nucleic acid according to claim 1.

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15. A cell containing an expression vector according to claim 13.
16. A process for recombinant production of a VDRR polypeptide, said process comprising expressing the nucleic acid of claim 1 in a suitable host cell.
- 5 17. The process according to claim 16, wherein the host cell is eukaryotic.
18. An isolated or recombinant VDRR polypeptide.
- 10 19. The isolated or recombinant VDRR polypeptide according to claim 18 comprising the amino acid sequence given in Fig. 4 or Fig. 8.
20. A pharmaceutical formulation comprising an isolated or recombinant VDRR polypeptide according to claims 18 or 19, and one or more therapeutically acceptable
- 15 excipients.
21. A method for identifying a ligand to a VDRR, by a cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay.
- 20 22. A method for identifying a substance for treatment of a condition affected by a VDRR polypeptide, comprising screening for an agonist or an antagonist of VDRR polypeptide signal transduction to be used for treating metabolic, proliferative or inflammatory conditions.
- 25 23. A VDRR polypeptide for use as a medicament.
24. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions.

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25. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesteremia or hyperlipoproteinemia.
- 5 26. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating osteoporosis, rheumatoid arthritis, benign and malign tumors, hyperproliferative skin disorders or hyperparathyroidism.
- 10 27. Use according to any of claims 24-26, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.
- 15 28. A method for treating metabolic, proliferative or inflammatory conditions comprising introducing into a mammal a nucleic acid vector according to claim 13 encoding for expression of a VDRR polypeptide and wherein said nucleic acid vector is capable of transforming a cell *in vivo* and expressing said polypeptide in said transformed cell.
- 20 29. A method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR signal transduction.
- 25 30. The method according to claim 29, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.

ABSTRACT

The present invention relates to novel vitamin D receptor related (VDRR) poly-
 5 peptides, and formulations containing the same. Nucleic acid sequences encoding the
 VDRR polypeptides, expression vectors containing such sequences and host cells trans-
 formed with such expression vectors are also disclosed, as are methods for the expression
 of the novel VDRR polypeptides of the invention. The invention further relates to VDRR
 polypeptides for use as medicaments, and use of substances affecting VDRR signal trans-
 10 duction for the manufacture of medicaments for treating metabolic, proliferative or inflam-
 matory conditions. The present invention also relates to methods for identifying clones
 encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods
 for identifying substances for treatment of conditions affected by a VDRR polypeptide.
 More specifically, the novel VDRR polypeptide can be the polypeptide designated
 15 VDRR γ , which may be regulated by any small chemical molecule similar in structure to
 known ligands for nuclear receptors.

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1 CCTCTGAAGG TTCTAGAATC GATAGTGAAT TCGTGGGACG GGAAGAGGAA
51 GCACTGCCTT TACTTCAGTG GGAATCTCGG CCTCAGCCTG CAAGCCAAGT
101 GTTCACAGTG AAAAAAGCAA GAGAATAAGC TAATACTCCT GTCCTGAACA
151 AGGCAGCGGC TCCTTGGTAA AGCTACTCCT TGATCGATCC TTTGCACCGG
201 ATTGTTCAAA GTGGACCCCA GGGGAGAAGT CGGAGCAAAG AACTTACCAC
251 CAAGCAGTCC AAGAGGCCCA GAAGCAAACC TGGAGGTGAG ACCCAAAGAA
301 AGCTGGAACC ATGCTGACTT TGTACACTGT GAGGACACAG AGTCTGTTCC
351 TGGAAAGCCC AGTGTCAACG CAGATGAGGA AGTCGGAGGT CCCCAAATCT
401 GCCGTGTATG TGGGGACAAG GCCACTGGCT ATCACTTCAA TGTCATGACA
451 TGTGAAGGAT GCAAGGGCTT TTTCAGGAGG GCCATGAAAC GCAACGCCCG
501 GCTGAGGTGC CCCTTCCGGA AGGGCGCCTG CGAGATCACC CGGAAGACCC
551 GGCACAGTG CCAGGCCTGC CGCCTGCGCA AGTGCCTGGA GAGCGGCATG
601 AAGAAGGAGA TGATCATGTC CGACGAGGCC GTGGAGGAGA GCGGGCCTT
651 GATCAAGCGG AAGAAAAGTG AACGGACAGG GACTCAGCCA CTGGGAGTGC
701 AGGGGCTGAC AGAGGAGCAG CGGATGATGA TCAGGGAGCT GATGGACGCT
751 CAGATGAAAA CCTTTGACAC TACCTTCTCC CATTCAAGA ATTTCCGGCT
801 GCCAGGGGTG CTTAGCAGTG GCTGCGAGTT GCCAGAGTCT CTGCAGGCCC
851 CATCGAGGGA AGAAGCTGCC AAGTGGAGCC AGGTCCGGAA AGATCTGTGC
901 TCTTTGAAGG TCTCTCTGCA GCTGCGGGGG GAGGATGGCA GTGTCTGGAA
951 CTACAAACCC CCAGCCGACA GTGGCGGGAA AGAGATCTTC TCCCTGCTGC
1001 CCCACATGGC TGACATGTCA ACCTACATGT TCAAAGGCAT CATCAGCTTT
1051 GCCAAAGTCA TCTCCTACTT CAGGGACTTG CCCATCGAGG ACCAGATCTC
1101 CCTGCTGAAG GGGGCCGCTT TCGAGCTGTG TCAACTGAGA TTCAACACAG
1151 TGTTC AACGC GGAGACTGGA ACCTGGGAGT GTGGCCGGCT GTCCTACTGC
1201 TTGGAAGACA CTGCAGGTGG CTTCCAGCAA CTTCTACTGG AGCCCATGCT
1251 GAAATTCCAC TACATGCTGA AGAAGCTGCA GCTGCATGAG GAGGAGTATG
1301 TGCTGATGCA GGCCATCTCC CTCTTCTCCC CAGACCGCCC AGGTGTGCTG
1351 CAGCACCGCG TGGTGGACCA GCTGCAGGAG CAATTCGCCA TTA CTCTGAA
1401 GTCCTACATT GAATGCAATC GGCCCCAGCC TGCTCATAGG TTCTTGTTC

Fig. 1

1451 TGAAGATCAT GGCTATGCTC ACCGAGCTCC GCAGCATCAA TGCTCAGCAC
1501 ACCCAGCGGC TGCTGCGCAT CCAGGACATA CACCCCTTTG CTACGCCCCCT
1551 CATGCAGGAG TTGTTTCGGCA TCACAGGTAG CTGAGCGGCT GCCCTTGGGT
1601 GACACCTCCG AGAGGCAGCC AGACCCAGAG CCCTCTGAGC CGCCACTCCC
1651 GGGCCAAGAC AGATGGACAC TGCCAAGAGC CGACAATGCC CTGCTGGCCT
1701 GTCTCCCTAG GGAATTCCTG CTATGACAGC TGGCTAGCAT TCCTCAGGAA
1751 GGACATGGGT GCCCCCCACC CCCAGTTCAG TCTGTAGGGA GTGAAGCCAC
1801 AGACTCTTAC GTGGAGAGTG CACTGACCTG TAGGTCAGGA CCATCAGAGA
1851 GGCAAGGTTG CCCTTTCCTT TAAAAGGCC CTGTGGTCTG GGGAGAAATC
1901 CCTCAGATCC CACTAAAGTG TCAAGGTGTG GAAGGGACCA AGCGACCAAG
1951 GATAGGCCAT CTGGGGTCTA TGCCACATA CCCACGTTTG TTCGCTTCCT
2001 GAGTCTTTTC ATTGCTACCT CTAATAGTCC TGTCTCCAC TTCCCACTCG
2051 TTCCCCTCCT CTTCCGAGCT GCTTTGTGGG CTCAAGGCCT GTACTCATCG
2101 GCAGGTGCAT GAGTATCTGT GGGAGTCCTC TAGAGAGATG AGAAGCCAGG
2151 AGGCCTGCAC CAAATGTCAG AAGCTTGGCA TGACCTCATT CCGGCCACAT
2201 CATCTGTGT CTCTGCATCC ATTTGAACAC ATTATTAAGC ACTGATAATA
2251 GGTAGCCTGC TGTGGGGTAT ACAGCATTGA CTCAGATATA GATCCTGAGC
2301 TCACAGAGTT TATAGTTAAA AAAACAAACA GAAACACAAA CAATTTGGAT
2351 CAAAAGGAGA AAATGATAAG TGACAAAAGC AGCACAAGGA ATTTCCCTGT
2401 GTGGATGCTG AGCTGTGATG GCAGGCACTG GGTACCCAAG TGAAGGTTCC
2451 CGAGGACATG AGTCTGTAGG AGCAAGGGCA CAACTGCAG CTGTGAGTGC
2501 GTGTGTGTGA TTTGGTGTAG GTAGGTCTGT TTGCCACTTG ATGGGGCCTG
2551 GGTTTGTTCC TGGGGCTGGA ATGCTGGGTA TGCTCTGTGA CAAGGCTACG
2601 CTGACAATCA GTTAAACACA CCGGAGAAGA ACCATTTACA TGCACCTTAT
2651 ATTTCTGTGT ACACATCTAT TCTCAAAGCT AAAGGGTATG AAAGTGCCCTG
2701 CCTTGTTTAT AGCCACTTGT GAGTAAAAAT TTTTTGCAT TTTCACAAAT
2751 TATACTTAT ATAAGGCATT CCACACCTAA GAACTAGTTT TGGGAAATGT
2801 AGCCCTGGGT TTAATGTCAA ATCAAGGCAA AAGGAATTAA ATAATGTACT
2851 TTTGGCTAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
2901 AAAAA

Fig. 1 (cont.)

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Evolutionary Neighbour-Joining Tree

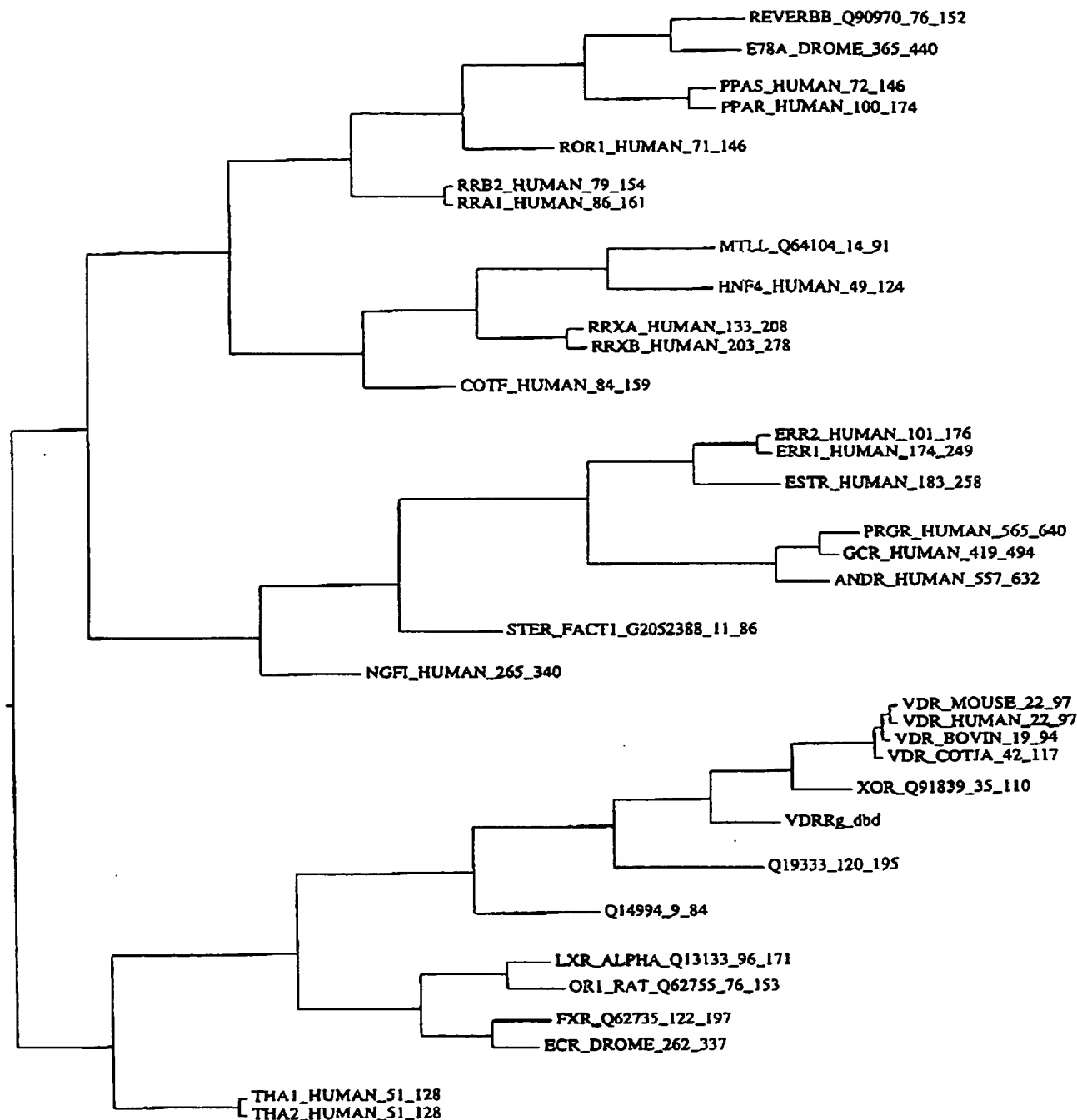


Fig. 2

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Evolutionary Neighbour-Joining Tree

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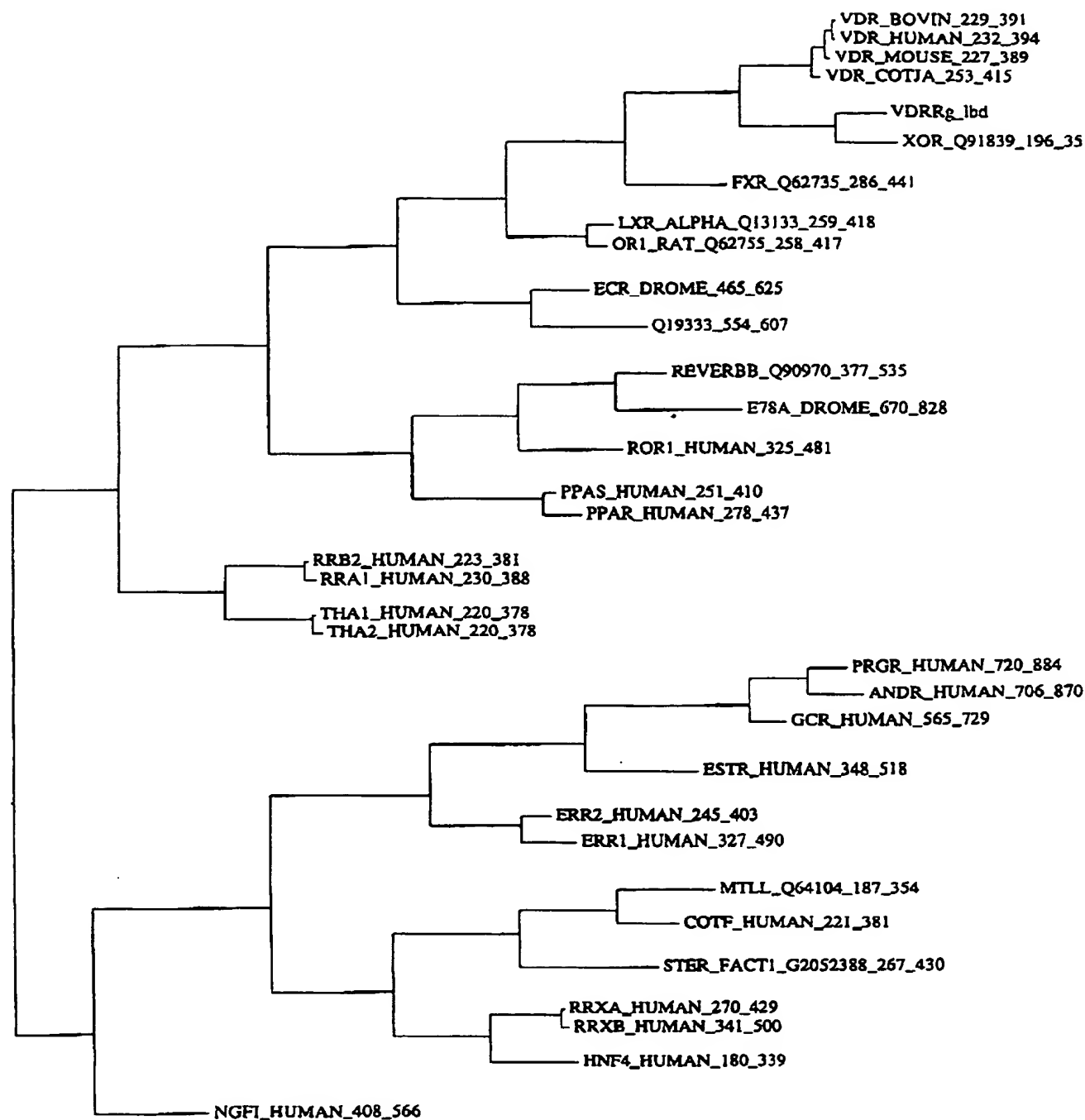


Fig. 3

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1 MEVRPKESWN HADFVHCEDT ESVP GKPSVN ADEEVGGPQI CRVCGDKATG
 51 YHFNVMTC EG CKGFFRRAMK RNARLRCPFR KGACEITRKT RRQCQACRLR
 101 KCLESGMKKE MIMSDEAVEE RRALIKRKKS ERTGTQPLGV QGLTEEQRMM
 151 IRELMDAQMK TFDTTFSHK NFRLPGVLSS GCELPESLQA PSREEAAKWS
 201 QVRKDLCSLK VSLQLRGEDG SVWNYKPPAD SGGKEIFSLL PHMADMSTYM
 251 FKGHSFAKV ISYFRDLPIE DQISLLKGAA FELCQLRFNT VFNAETGTWE
 301 CGRLSYCLED TAGGFQQLLL EPMLKFHYML KKLQLHEEEY VLMQAISLFS
 351 PDRPGVLQHR VVDQLQEQA ITLKSYTECN RPQPAHRFLF LKIMAMLTTEL
 401 RSINAQHTQR LLRIQDIHPF ATPLMQELFG ITGS

Fig. 4

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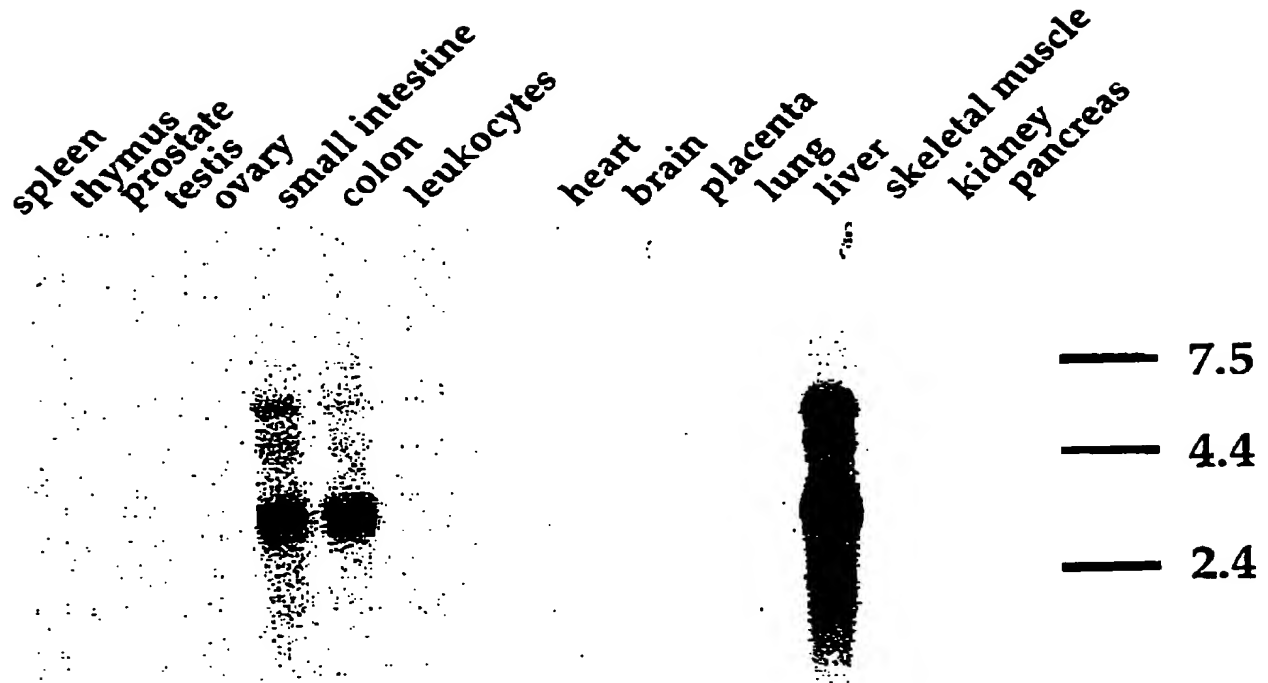


Fig. 5

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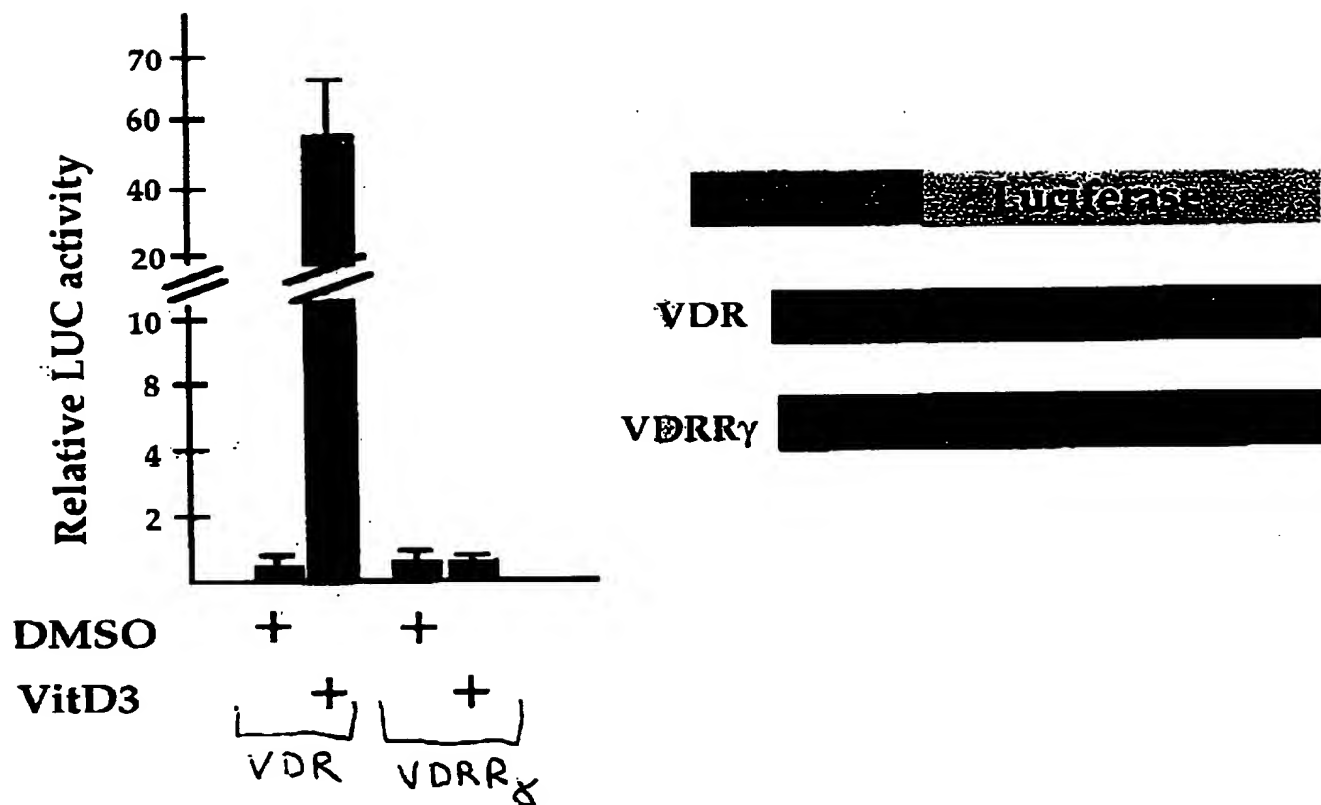


Fig. 6

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TGAATTGGTGGGCGCTGGCTGGGTTAGTGTCTGGCAGCCCCC 40
 TGAGGCCAAGGACAGCAGCATGACAGTCACCAGGACTCAC 80
 CACTTCAAGGAGGGGTCCCTCAGAGCACCTGCCATACCCC 120
 TGCACAGTGTCTGGCGCTGAGTGGCTTCAAACCATCCAAG 160
 AGGCCCAGAAGCAAACCTGGAGGTGAGACCCAAAGAAAGC 200
 TGGAAACCATGTCTGACTTTGTACACTGTGAGGACACAGAGT 240
 CTGTTCCTTGGAAAGCCCAGTGTCAACGCAGATGAGGAAGT 280
 CGGAGGTCCCCAAATCTGCCGTGTATGTGGGGACAAGGCC 320
 ACTGGCTATCACTTCAATGTTCATGACATGTGAAGGATGCA 360
 AGGGCTTTTTCAGGAGGGCCATGAACGCCAACGCCCGGCT 400
 GAGGTGCCCCCTTCCGGAAGGGCGGCTGCCGAGATCACCGG 440
 AAGACCGGGGACAGTGGCAGGCGCTGCCGCGCTGCCCAAGT 480
 GCCTGGAGAGCGGCATGAAGAAGGAGATGATCATGTCCGA 520
 CGAGGCGGTGGAGGAGAGGGCGGGCTTIGATCAAGCGGAAG 560
 AAAAGTGAACGGACAGGGACTCAGCCACTGGGAGTGCAGG 600
 GGCTGACAGAGGAGCAGCGCATGATGATCAGGGAGCTGAT 640
 GGACGCTCAGATGAAAACCTTTTGACACTACCTTCTCCCAT 680
 TTCAAGAATTTCCGGCTGCCAGGGGTGCTTAGCAGTGGCT 720
 GCGAGTTGCCAGAGTCTCTGCGAGGCCCCATCGAGGGAAGA 760
 AGCTGCCAAGTGGAGCCAGGTCCGGAAAGATCTGTGCTCT 800
 TTGAAGGCTCTCTCTGCAGCTGCCGGGGGAGGATGGCAGTG 840
 TCTGGAACTACAAACCCCCAGCCGACAGTGGCGGGGAAGA 880
 GATCTTCTCCCTGCTGCCCCACATGGCTGACATGTCAAC 920
 TACATGTTCAAGGCATCATCAGCTTTGCCAAGTTCATCT 960
 CCTACTTTCAGGGACTTGGCCATCGAGGACCAGATCTCCCT 1000
 GCCTGAAGGGGGCGGCTTTCCGAGCTGTGTCAACTGAGATT 1040
 AACACAGTGTTCACCGCGGAGACTGGAACCTGGGAGTGTG 1080
 GCGGCTGTCTACTGCTTTGGAAGACACTGCAGGTGGCTT 1120
 CCAGCAACTTCTACTGGAGCCCATGCTGAAATTCCACTAC 1160
 ATGCTGAAGAAGCTGCAGCTGCATGAGGAGGAGTATGTGC 1200
 TGATGCAGGCCATCTCCCTCTCTTCCCCAGACCGCCAGG 1240
 TGTGCTGCAGCACCGCGTGGTGGACCAGCTGCAGGAGCAA 1280
 TTGGCCATTACTCTGAAGTCCCTACATTTGAATGCAATCGGC 1320
 CCCAGCCTGCTCATAGGTTCCTTGTTCCTGAAGATCATGGC 1360
 TATGCTCAACGAGCTCCGCAGCATCAATGCTCAGCACACC 1400
 CAGCGGCTGCTGGCATCCAGGACATACACCCCTTTGCTA 1440

Fig. 7

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CGCCCCCTCATGCGAGGAGTGTGTCGGCATCACAGGTAGCTG 1480
 AGCGGCTGCCCCTIGGGTGACACCTCCGAGAGGCAGCCAGA 1520
 CCCAGAGCCCCCTCTGAGCGGCCACTCCCCGGGCCAAGACAGA 1560
 TGGACACTGCCAAGAGCGGACAATGCCCTGCTGGCTGTGTC 1600
 TCCCTAGGGAATTCCCTGCTATGACAGCTGGCTAGCATTC 1640
 TCAGGAAGGACATGGGTGCCCCCACCCCCAGTTCAGTCT 1680
 GTAGGGAGTGAAGCCACAGACTCTTACGTGGAGAGTGCAC 1720
 TGACCTGTAGGTGAGGACCATCAGAGAGGCAAGGTTGCCC 1760
 TTTCCTTTTAAAAGGCCCTGTGGTCTGGGGAGAAATCCCT 1800
 CAGATCCCACTAAAGTGTCAAGGTGTGGAAGGGACCAAGC 1840
 GACCAAGGATAGGCCATCTGGGGTCTATGCCACATAACC 1880
 ACGTTTGTTCGCTTCCCTGAGTCTTTTCATTGCTAOCCTCA 1920
 ATAGTCCCTGCTCCCACTTCCCACTCGTTCCCCCTCCTCTT 1960
 CCGAGCTGCTTTGTGGGCTCAAGGCCTGTACTCATCGGCA 2000
 GGTCATGAGTATCTGTGGGAGTCCCTCTAGAGAGATGAGA 2040
 AGCCAGGAGGCTGCACCAAAATGTGAGAAGCTTGGCATGA 2080
 CCTCATTCGCGGCCACATCATTCTGTGTCTCTGCATCCATT 2120
 TGAACACATTATTAAGCACTGATAATAGGTAGCCTGCTGT 2160
 GGGGTATACAGCATTTGACTCAGATATAGATCCCTGAGCTCA 2200
 CAGAGTTTATAGTTAAAAAACAAACAGAAACACAAACAA 2240
 TTTGGATCAAAAGGAGAAATGATAAGTGACAAAAGCAGC 2280
 ACAAGGAATTTCCCTGTGTGGATGCTGAGCTGTGATGGCA 2320
 GGCCTGGGTACCCAAGTGAAGGTTCCCGAGGACATGAGT 2360
 CTGTAGGAGCAAGGGCACAACTGCAGCTGTGAGTGGTG 2400
 TGTGTGATTTGGTGTAGGTAGGTCTGTGTGGCACTTGTATG 2440
 GGGCTGGGTGTGTCTCTGGGGCTGGAATGCTGGGTATGC 2480
 TCTGTGACAAGGCTACGCTGACAATCAGTTAAACACACCG 2520
 GAGAAGAACCATTTACATGCACCTTATATTTCTGTGTACA 2560
 CATCTATTCTCAAAGCTAAAGGGTATGAAAGTGCCCTGCT 2600
 TGTTTATAGCCACTTGTGAGTAAAAATTTTTTTTGCATTTT 2640
 CACAAATTATACTTTATATAAGGCATTCCACACCTAAGAA 2680
 CTAGTTTIGGGAATGTAGCCCTGGGTTTAATGTCAAATC 2720
 AAGGCAAAAGGAATTAAATAATGTACTTTTGGCTAAAAAA 2760
 AA 2800
 AA 2802

Fig. 7 (cont.)

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MTVTRTHHFKEGSLRAPAIPLHSAAAELASNHPRGPEANL 40
 EVRPKESWNHADDFVHCEDTESVPGKPSVNADEEVGGPQIC 80
 RVC GDKATGYHFNVMTCEGCKGFFRRAMKRNARLRCPFRK 120
 GACEITRKTRRQCQACRLRKCLESGMKKEMIMSDEAVEER 160
 RALIKRKKCERTGTQPLGVQGLTEEQRMMIRELMDAQMK 200
 FDTTFSHFKNFRLPGVLSSGCELPESLQAPSREEAAKWSQ 240
 VRKDLCSLKVSLQLRGEDGSVWNYKPPADSGGKEIFSLLP 280
 HMADMSTYMFKGIISFAKVISYFRDLP TEDQISLLKGA AF 320
 ELCQLRFNTVFNAETGTWECGRLSYCLEDTAGGFQQLLLE 360
 PMLKFHYMLKKLQLHEEEYVLMQAISLFS PDRPGVLQHRV 400
 VDQLQE QFAITLKS YIECNRPQPAHRFLFLKIMAMLT ELR 440
 SINAQHTQRLRLRIQDIHPFATPLMQELFGITGS. 474

Fig. 8

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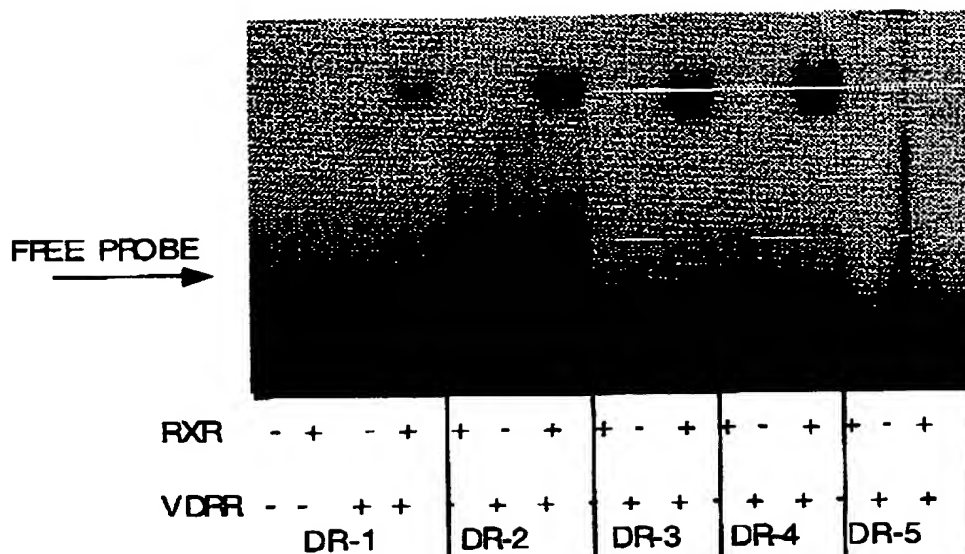


Fig. 9

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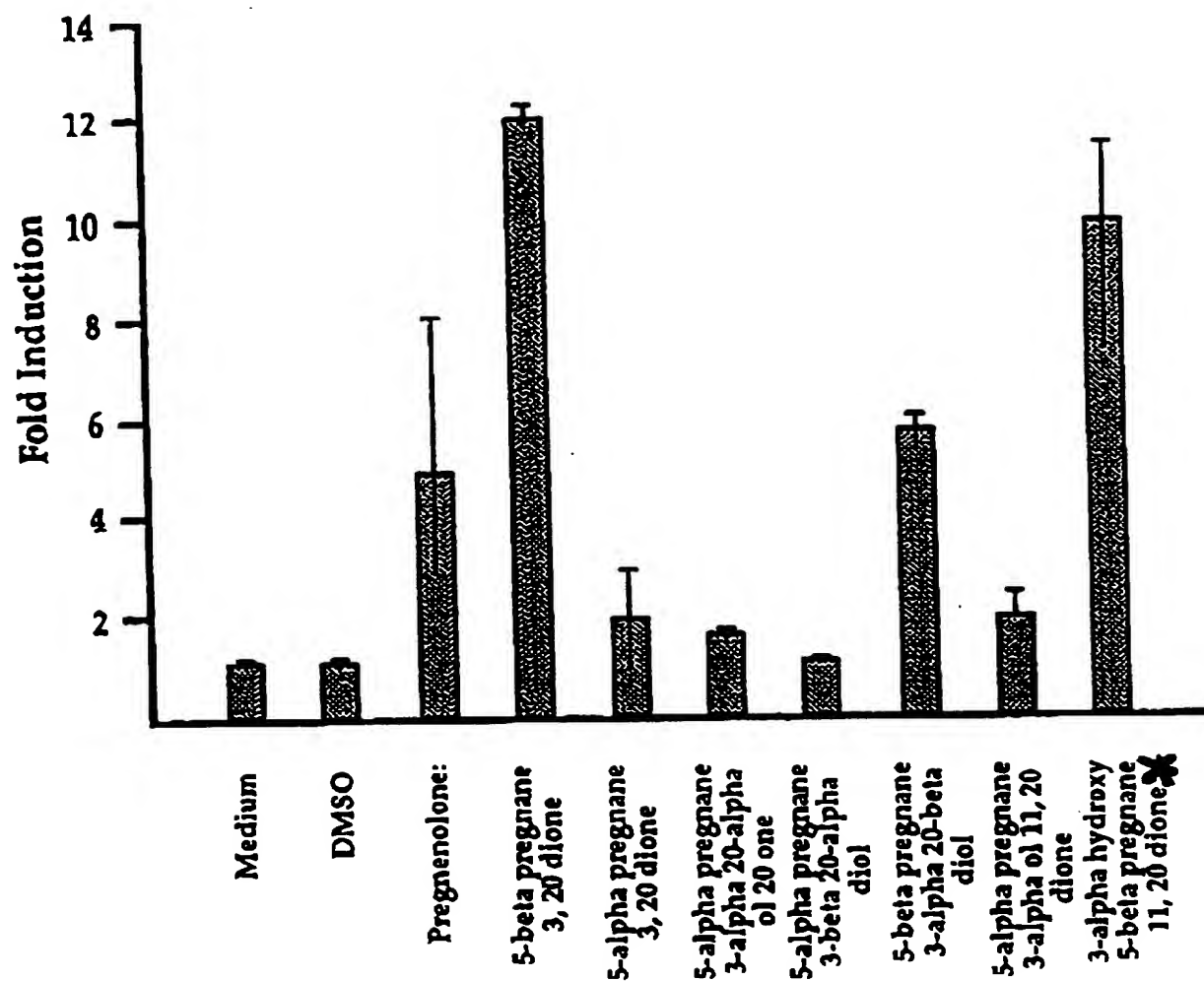


Fig. 10

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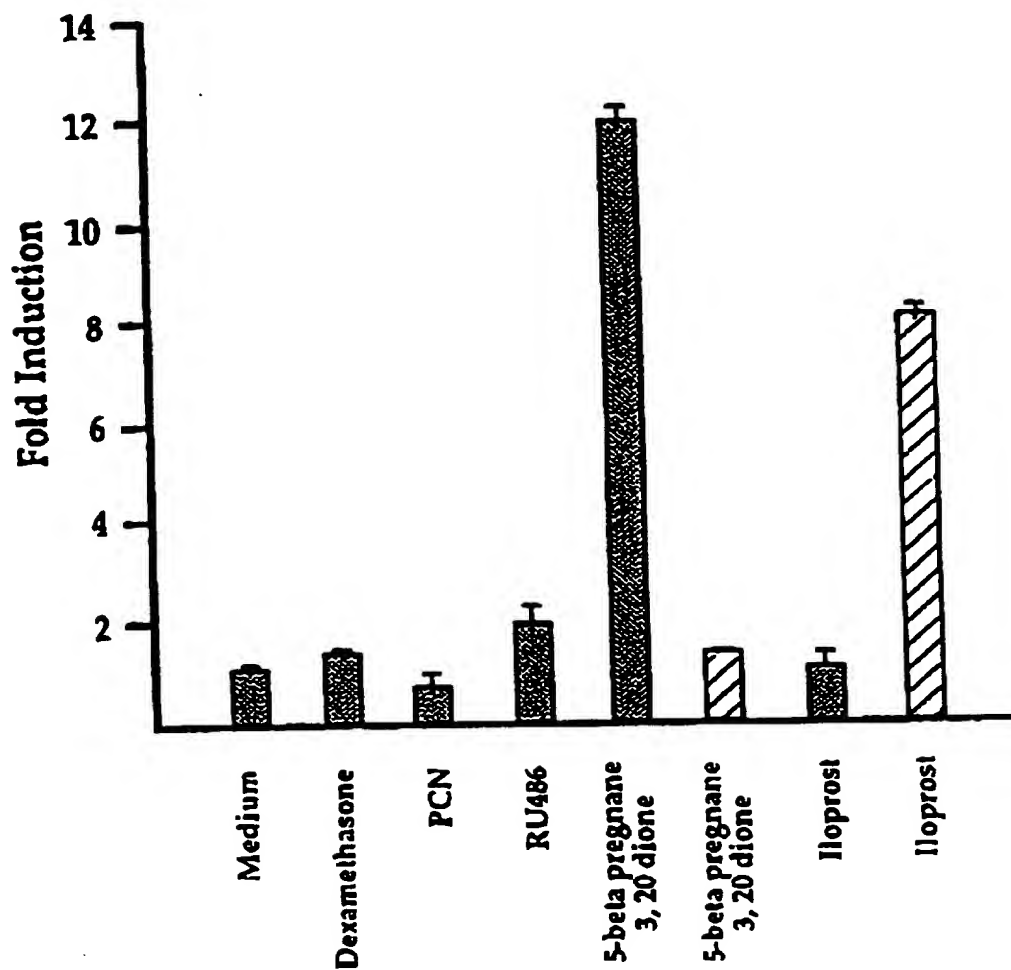


Fig. 11